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The Effect of a Probiotic and a Prebiotic on Productive Performance, Egg Quality, Blood Chemistry and Intestinal Morphology of Laying Japanese Quails

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Abstract

The use of alternative feed additives, such as prebiotics, probiotics, or their combination (synbiotics), can help control pathogens and enhance the physiological performance of poultry. This study evaluated the effects of dietary probiotic and prebiotic supplementation on productive performance, egg quality, blood parameters, gut morphology, carcass traits, and cecal microflora in Japanese laying quails. A total of 196 laying quails, 22 weeks old, were randomly assigned to seven dietary treatments with seven replicates of four birds each for 56 days: basal control diet (C), 1% whey powder (W), whey powder + 1% dried mealworm (WM), whey powder + *Lactobacillus plantarum* (WB), whey powder + commercial probiotic (100 g/ton) (WP), mealworm + *Lactobacillus plantarum* + whey powder (MBW), and mealworm + commercial probiotic + whey powder (MPW). Egg production, feed conversion ratio, and most egg quality traits were not significantly affected by dietary treatments ($P>0.05$). Feed intake was significantly reduced in the W, WB, MBW, and MPW groups compared with the control ($P<0.05$). Gut morphology was significantly altered, with supplemented diets generally increasing duodenal villus length, villus width, and villus-to-crypt ratio compared with the control ($P<0.05$). Whey supplementation increased serum protein, phosphorus, and triglyceride levels, whereas combining whey with probiotics and prebiotics mitigated the rise in triglycerides ($P<0.05$). No significant differences were observed in carcass yield, internal organ weights, or cecal *Lactobacillus* and *Escherichia coli* counts ($P>0.05$). In conclusion, dietary supplementation with prebiotic and probiotic sources, whether administered individually or in combination, improved specific gut morphological traits and modulated selected blood biochemical parameters without compromising performance or egg quality in laying Japanese quails.

Introduction

The poultry industry is one of the most significant sectors in the global meat market due to its capacity to produce meat and eggs at relatively low cost compared with other livestock systems. For decades, antibiotics were widely used in poultry production for disease prevention, treatment, and as growth promoters (Acosta *et al.*, 2025). However, concerns over antibiotic residues in poultry products and the rise of antimicrobial resistance have prompted restrictions on their use (Abou-Jaoudeh *et al.*, 2024). This has driven the search for alternative feed additives that can control intestinal pathogens and improve bird health without compromising productivity.

Among the most studied alternatives are probiotics, prebiotics, and their combination, known as synbiotics (Pandey *et al.*, 2015). Probiotics are live beneficial microorganisms, such as certain strains of *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces*

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boulardii, which can improve nutrient digestion, modulate gut microflora, and enhance intestinal integrity, thereby supporting overall performance (Khan and Naz, 2013; Yang *et al.*, 2025). Multi-strain probiotics often show greater efficacy than single-strain products because of synergistic interactions between strains (Kazemi *et al.*, 2019).

Prebiotics are non-digestible feed components, often dietary fibers, that selectively stimulate the growth and activity of beneficial gut bacteria such as lactobacilli and bifidobacteria (Yang *et al.*, 2025). Examples include whey powder and chitin (Ricke, 2021). By supporting probiotic populations, prebiotics help suppress harmful microorganisms and improve gut health (Yang *et al.*, 2025). Synbiotics combine probiotics and prebiotics to maximize these benefits, with evidence suggesting that synergistic combinations can be more effective than either component alone in stimulating beneficial microbiota and enhancing immunity (Pandey *et al.*, 2015; Slizewska *et al.*, 2020).

In recent years, insect-derived feed ingredients have attracted attention as sustainable protein and functional feed sources. Species such as black soldier fly (*Hermetia illucens*) and yellow mealworm (*Tenebrio molitor*) are of particular interest due to their high nutritional value, low production costs, and minimal environmental impact (Belhadj Slimen *et al.*, 2023). Mealworm is also rich in chitin, a natural prebiotic that can modulate gut microbiota (Belhadj Slimen *et al.*, 2023; Dalle Zotte *et al.*, 2024). In addition, bacterial species such as *Bacillus subtilis*—commonly used in poultry diets—produce chitinase, contributing to chitin degradation while acting as a robust probiotic due to their spore-forming ability and tolerance to processing conditions (Latorre *et al.*, 2014; Jazi *et al.*, 2018).

Whey powder, a dairy by-product containing high-quality proteins and ~70% lactose (dry matter basis), can also function as a prebiotic source (Madureira *et al.*, 2007). Although poultry cannot directly digest lactose, fermentation by beneficial gut bacteria produces short-chain fatty acids (SCFA) such as butyrate, propionate, and acetate, which support gut health and pathogen control (Pineda *et al.*, 2018; Ricke, 2021).

Given the functional potential of these feed ingredients, this study aimed to evaluate the effects of mealworm larvae powder and whey powder (as prebiotics) and probiotics, administered individually or in combination, on productive performance, blood serum parameters, gut morphology, and cecal microflora of laying Japanese quails.

Materials and Methods

Birds and dietary treatments

This experiment was conducted with the approval of the Ethics Committee of Razi University, Kermanshah, Iran (Approval Code: IR.RAZI.REC.1400.091), in accordance with institutional guidelines for the care and use of animals. A total of 196 Japanese quails, 22 weeks of age, were randomly assigned to seven dietary treatments with seven replicates per treatment and four birds per replicate, for a 56-day trial. Quails were housed in wire battery cages ($50 \times 40 \times 50$ cm^3) equipped with feeders and nipple drinkers. Environmental conditions were maintained at 20–22 °C, 50–55% relative humidity, and a photoperiod of 16 hours light: 8 hours dark. Quails were vaccinated during early life according to standard health protocols, and from the beginning of the experiment, they received a monthly Newcastle disease (B1 strain) booster via drinking water. *Lactobacillus plantarum* (4×10^9 CFU/kg feed), obtained from the Iranian Biological Resource Center, was cultured in MRS broth (Vandidaz Co., Tehran, Iran) and incorporated into the diets daily. A commercial probiotic (Parsi Lact; Pardis Roshd Mehregan Co., Fars, Iran) containing *Bacillus subtilis* (2×10^9 CFU/g), *Bacillus ligiiformis* (2×10^9 CFU/g), *Bacillus coagulans* (2×10^{11} CFU/g), *Lactobacillus rhamnosus* (2×10^9 CFU/g), *Enterococcus faecium* (2×10^{10} CFU/g), and *Lactobacillus plantarum* (2×10^9 CFU/g) was included at 100 g/ton of feed. The treatment levels were selected based on previous experimental findings and relevant literature to ensure biologically meaningful and safe inclusion rates for the quails. Dried whey powder, obtained from Aneed Company, Mashhad, Iran, was used as a prebiotic source, and its composition is presented in Table 1. All diets were formulated to meet quail nutrient requirements according to NRC (1994) recommendations. The proximate composition of the experimental diets, including crude protein, crude fiber, crude fat, ash, calcium, and phosphorus, was analyzed according to AOAC (2005) methods. Ingredient composition and calculated nutrient values for each experimental diet are shown in Table 2. Experimental treatments include: a control corn-SBM (C); corn-SBM diet containing 1% dried whey powder (W); corn-SBM diet containing 1% dried whey powder and 1% mealworm powder (M); corn-SBM diet containing 1% dried whey powder and 4×10^9 per kg of diet *Lactobacillus* bacteria (B); corn-SBM diet containing 1% dried whey powder and 100 g/ton Parsi Lact commercial probiotic feed (P); corn-SBM diet containing 1% dried whey powder, 1% mealworm powder and 4×10^9 per kg of diet *Lactobacillus* bacteria (MBW), corn-SBM diet containing 1% dried whey powder, 1% mealworm powder and 100 g/ton Parsi Lact commercial probiotic (MPW).

Table 1: Analysis of chemical compounds of whey powder

Chemical analysis	Amount % on dry matter basis
Dry matter	92.3
Crude protein	20.1
Total fat	3.50
Ash	10.5
Sodium	1.68
Calcium	0.42
Phosphorus	0.56
Potassium	1.60

Table 2: Ingredients and calculated composition of the experimental basal diets

Ingredient, %	Control	Mealworm	Whey	Mealworm + Whey
Mealworm	-	1	-	1
Whey	-	-	1	1
Corn	51.66	50.99	51.33	50.66
Soybean meal	36.94	35.49	36.98	35.52
Soybean oil	2.99	2.99	2.99	2.99
Wheat bran	0.64	1.79	-	1.15
Dicalcium phosphate	1.10	1.07	1.10	1.08
Calcium carbonate	2.80	2.80	2.80	2.80
Oyster	2.83	2.85	2.8	2.82
DL-Methionine	0.17	0.16	0.17	0.16
NaCl	0.32	0.32	0.29	0.28
Vitamin premix [1]	0.25	0.25	0.25	0.25
Mineral premix [2]	0.25	0.25	0.25	0.25
L-Threonine	0.05	0.04	0.04	0.04
Calculated values (%, unless mentioned)				
Metabolizable energy (Kcal/kg)	2900	2900	2900	2900
Crude protein	20.9	20.9	20.9	20.9
Ether extract	4.97	5.19	4.94	5.16
Crude fiber	3.79	3.95	3.72	3.8
Calcium	2.5	2.5	2.5	2.5
Available phosphorus	0.35	0.35	0.35	0.35
Sodium	0.15	0.15	0.15	0.15
Lysine	1.01	1.00	1.01	1.01
Cysteine + Methionine	0.75	0.75	0.75	0.75
Threonine	0.74	0.74	0.74	0.74

[1] Vitamin supplied per kg of diet: antioxidant, 40000mg; biotin, 40mg; Acid pantothenic, 1200mg; D_3, 800000IU; B_{12}, 60mg; folic acid, 52mg; K_3, 800mg; niacin, 720mg; pyridoxine, 1200mg; acid folic, 400mg; A, 2600000IU; B_2, 2640mg; B_3, 1200mg; E, 7200mg; choline chloride, 100000mg.

[2] Mineral supplied per kg of diet: Se, 80mg; Cu, 4000mg; Fe, 20000mg; Mn, 40000mg; I, 400 mg; Zn, 33880mg.

Preparation of mealworm

Mealworms were reared on a wheat bran diet for 16–18 weeks. Fresh vegetables, including carrots and potatoes, were provided at two-day intervals to supply essential moisture and promote optimal growth. Larvae were harvested just prior to pupation to ensure full development. After harvesting, all impurities were removed, and the larvae were fasted for two days to empty their digestive tracts. The cleaned larvae were then frozen at −21°C for 48 hours, followed by drying in an incubator at 60°C for 24 hours. Once dried, the larvae were ground to a uniform powder for inclusion in quail diets. The resulting mealworm powder was analyzed for proximate composition, including crude protein, ether extract, crude fiber, crude ash, calcium, and phosphorus, following standard AOAC (2005) procedures. The amino acid profile of the larvae was determined using high-performance liquid chromatography (HPLC), and the chitin content was calculated using the formula (Marono *et al*., 2015) (Table 3):

$$Chitin = ash - free\ acid - detergent\ fiber\ (\%) - acid - detergent\ insoluble\ protein\ (\%)$$

Table 3: Analysis of chemical compounds and amino acids of yellow flour *Tenebrio molitor* larvae (as percent of dry matter basis)

Analyzed compositions	Amounts
Dry matter	97.1
Crude protein	52.61
Ether extract	28.3
Crude ash	6.78
Crude fiber	7.43
Chitin	5.6
Calcium	3.50
Phosphorus	6.60
Methionine	0.667
Cysteine	0.434
Lysine	2.748
Threonine	1.899
Arginine	2.591
Iso lucien	2.769
Lucien	3.931
Valine	2.977
Histidine	1.542
Phenylalanine	1.748
Glycine	2.524
Serine	2.164
Proline	3.23
Alanine	3.24
Aspartic acid	3.97
Glutamic acid	5.81

Productive performance

Following a one-week acclimation period to the designated diets, data collection commenced. Body weight (BW) was measured at the beginning and end of the study. Daily feed intake (FI, g/bird) was ascertained through a method where the initial amount of feed offered was subtracted from the weight of the remaining feed in the feeder. Each day, the eggs laid within each replicate were counted and weighed (EW) using an electronic scale, with the average weight recorded. Upon completion of each four-week experimental period, calculations were performed to determine the egg mass (EM), feed conversion ratio (FCR), and egg production (EP). The FCR (g feed/g egg) was obtained by dividing the total g of feed consumed by the total g of eggs produced. EM was calculated by multiplying the average EW by the number of hens laying eggs on a given day. Mortality rates were documented throughout the experiment to allow for data adjustments. The percentage of EP was calculated based on the number of chickens available per day and using the following formula.

$$Production\ of\ quail\ eggs\ (hen\ day) = \frac{(Total\ number\ of\ eggs\ produced\ by\ Quail)}{(number\ of\ hen\ days)\ \times 100}$$

Egg quality characteristics

At the end of the experiment, two eggs were collected from each replicate over three consecutive days, resulting in four eggs per replicate for each sampling period. Egg quality traits, including Haugh unit (HU), albumen height, shell percentage, shell thickness, albumen percentage, and yolk percentage, were measured. Utilizing a dial and pipe gauge (Ozaki MFG. Co., Tokyo, Japan), measurements of shell thickness was obtained at three designated locations on the egg: the air cell, equator, and sharp end. Subsequently, the average of these measurements was calculated. Shell percentage was calculated based on the individual weight of each egg and the weight of the shell itself. Eggs were cracked open, and the yolk was separated from the albumen. The yolk weight and albumen weight were then measured as a percentage of the whole EW. To calculate the Haugh unit, the weight of each quail egg and the height of its albumen (measured by a micrometer (Mitutoyo, 0.01 mm, Japan)) were first determined. Then, the Haugh unit values were calculated for each quail egg using the following formula (Eisen *et al.,* 1962):

$$Haugh\ unit = 100 \times log10\ (H + 7.57 - 1.7\,W^{0.37})$$

Where H is the albumen height in mm, and W is the quail EW in g.

Blood sample collection

Two blood samples per replicate were collected from the brachial vein of the wing into syringes containing EDTA. After centrifugation at 1500 g for 15 minutes at room temperature, the serum fraction was separated, frozen, and stored at –20 °C until analysis. Blood serum metabolites, including glucose, total protein, albumin, triglyceride, cholesterol, low-density lipoprotein cholesterol (LDL-c), High-density lipoprotein cholesterol (HDL-c), calcium, and phosphorus, were determined using commercial diagnostic kits (Pars Azmun, Tehran, Iran). These kits rely on standard biochemical methods: enzymatic colorimetric assays for glucose, triglycerides, cholesterol, and lipoproteins; the biuret method for total protein; the Bromocresol green dye-binding method for albumin; and colorimetric methods for calcium and phosphorus. All assays were performed following the manufacturer's instructions.

Intestinal morphology

Intestinal morphology was assessed following the methods described by Iji *et al.* (2001). Mid-intestinal segments (approximately 5 mm) from the duodenum, jejunum, and ileum were surgically excised and fixed in 10% buffered formalin for 72 hours. Samples were then rinsed with saline, and tissue sections (6 µm thick) were prepared using a rotary microtome (Leica RM 2145, GMI Inc., USA). Sections were stained with hematoxylin and eosin and examined under a light microscope. Morphometric parameters, including villus height (from the villus–crypt junction to the villus tip), crypt depth (from the villus–crypt junction to the crypt base), and the villus height-to-crypt depth ratio, were measured using Image-Pro Plus v4.5 software (Media Cybernetics, USA).

Cecal microbial analysis

At the conclusion of the experiment, two quail per pen were aseptically euthanized, and their caecal contents were immediately collected and transferred to sterile plates in the laboratory. One gram of the sample was mixed with 9 mL of sterile physiological saline (0.9% NaCl) to obtain a 1:10 (10^{-1}) dilution. Serial dilutions were prepared up to 10^{-5}. *Lactobacillus* spp. were cultured on MRS agar (Merck, Germany) and *Escherichia coli* on MacConkey agar (Merck, Germany). For each dilution, 250 µL was plated and incubated at 37 °C for 48 h (MRS) or 24 h (MacConkey). Colony-forming units (CFU) per gram of sample were calculated by multiplying the plate colony count by the respective dilution factor.

All microbiological procedures (media preparation, plating, incubation, and colony counting) were conducted under sterile conditions within a microbiology hood. It should be noted that these culture-based methods quantify only the culturable fraction of the gut microbiota; molecular approaches such as 16S rRNA gene sequencing would provide a more comprehensive characterization of the microbial community.

Internal organ weights

Thirty-week-old quails were deprived of food for two hours. Two birds from each pen were selected based on their average weight, then weighed and euthanized aseptically. The liver, heart, and gizzard were weighed and expressed as a percentage of the bird's live body weight.

Statistical analyses

The data were analyzed using SAS software (version 9.4; SAS Institute Inc., 2015) following a completely randomized design. The general linear model (GLM) procedure was applied, and Tukey's multiple range test was used to compare means at a significance level of $P< 0.05$.

$$Y_{ij} = \mu + T_i + e_{ij}$$

In this formula, Y_{ij} was the value of each observation, μ was the average of the whole experiment, T_i was the treatment effect, and e_{ij} was the experimental error.

Results

Productive performance

The results in Table 4 show the effects of dietary treatments on quail performance. Over the entire trial period (22–30 weeks), EP and FCR were not significantly influenced by any of the experimental diets ($P>0.05$). From weeks 22 to 26, FI was significantly lower in the WB and MPW treatments compared with the control ($P< 0.05$). During weeks 26 to 30, and across the whole 22–30 week period, FI was significantly reduced in the W, WB, MBW, and MPW treatments compared with the control ($P< 0.05$).

Egg quality traits

The effects of the experimental treatments on egg quality traits are shown in Tables 5, 6, and 7, corresponding to the age periods of 26 weeks, 30 weeks, and the mean age of 26 and 30 weeks, respectively. No significant differences ($P>0.05$) were observed between the control and experimental treatments across any of these periods.

Table 4: The effect of experimental treatments on the productive performance of laying quails

	Egg production (%)			Feed intake (g/day/bird)			FCR (feed/g egg)		
	22-26w	26-30w	22-30w	22-26w	26-30w	22-30w	22-26w	26-30w	22-30w
C[1]	86.7	84.4	85.5	29.9 [a]	30.4 [a]	30.1 [a]	2.80	2.89	2.85
W[2]	91.5	86.5	88.9	29.6 [ab]	28.3 [bc]	28.9 [bc]	2.56	2.60	2.56
WM[3]	88.5	89.8	89.2	29.4 [ab]	29.3 [ab]	29.4 [ab]	2.64	2.65	2.64
WB[4]	86.6	86.8	86.7	28.8 [c]	27.8 [c]	28.3 [c]	2.71	2.59	2.65
WP[5]	88.1	84.6	86.3	29.4 [abc]	29.1 [abc]	29.2 [ab]	2.68	2.79	2.73
MBW[6]	91.8	87.8	89.8	29.6 [ab]	28.6 [bc]	29.1 [bc]	2.66	2.58	2.61
MPW[7]	88.6	83.2	85.8	29.1 [bc]	27.8 [c]	28.5 [bc]	2.64	2.63	2.63
P values	0.668	0.720	0.718	0.001	0.001	0.001	0.503	0.124	0.173
SEM[8]	2.57	2.90	2.28	0.413	0.896	0.603	0.081	0.089	0.075

[1] C=control diet; [2] W= 1% of whey powder; [3] WM= 1% whey powder + 1% dried mealworm; [4] WB= 1% whey powder + *Lactobacillus plantarum* bacteria at the rate of 4 × 10⁹ CFU/kg; [5] WP= 1% whey powder + 100g/ton Parsi Lact commercial probiotic (including *Bacillus coagulans*, *Enterococcus faecium*, *Bacillus subtilis*, *Bacillus ligiiformis*,, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*) in the amount of 100 grams per ton of feed; [6] MBW= 1% mealworm + *Lactobacillus plantarum* + 1% whey powder; [7] MPW= 1% mealworm + commercial probiotic + 1% whey powder; [8] Standard error of mean.

Table 5: The effect of experimental treatments on egg quality traits of laying quails at 26 weeks of age

Parameters	Treatments								
	C[1]	W[2]	WM[3]	WB[4]	WP[5]	MBW[6]	MPW[7]	P values	SEM[8]
Egg weight (g)	13.1	13.4	13.4	13.2	13.1	13.8	13.2	0.553	0.239
Shell thickness (mm)	0.22	0.225	0.221	0.239	0.221	0.218	0.229	0.434	0.004
Albumen height(mm)	5.1	5.10	5.10	5.09	5.09	5.10	5.10	0.550	0.007
White (%)	57.9	56.7	56.6	58.1	58.7	57.8	57.7	0.245	0.643
Yolk (%)	33	33.4	33.8	32.2	32.00	32.8	32.9	0.324	0.569
Shell (%)	9.14	9.9	9.59	9.76	9.24	9.45	9.31	0.249	0.241
Haugh unit	91.7	91.6	91.4	91.7	91.5	91.8	91.6	0.297	0.106
Number of eggs	24.3	26.3	24.8	24.8	24.7	28.1	24.8	0.052	0.872
Egg mass (g/bird)	10.9	11.8	11.3	10.8	11.1	11.3	11.3	0.481	0.332

[1] C=control diet; [2] W= 1% of whey powder; [3] WM= 1% whey powder + 1% dried mealworm; [4] WB= 1% whey powder + *Lactobacillus plantarum* bacteria at the rate of 4 × 10⁹ CFU/kg; [5] WP= 1% whey powder + 100g/ton Parsi Lact commercial probiotic (including *Bacillus coagulans*, *Enterococcus faecium*, *Bacillus subtilis*, *Bacillus ligiiformis*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*) in the amount of 100 grams per ton of feed; [6] MBW= 1% mealworm + *Lactobacillus plantarum* + 1% whey powder; [7] MPW= 1% mealworm + commercial probiotic + 1% whey powder; [8] Standard error of mean.

Table 6: The effect of experimental treatments on egg quality traits of laying quails at 30 weeks of age

Parameters	Treatments								
	C[1]	W[2]	WM[3]	WB[4]	WP[5]	MBW[6]	MPW[7]	P values	SEM[8]
Egg weight (g)	12.4	12.7	12.6	12.4	12.3	12.4	12.9	0.347	0.208
Shell thickness (mm)	0.218	0.226	0.223	0.215	0.219	0.228	0.223	0.094	0.003
White height (mm)	5.05	5.05	5.03	5.06	5.04	5.06	5.10	0.084	0.168
White (%)	58.2	57.2	56.1	57.1	58.4	56.5	56.6	0.125	0.589
Yolk (%)	33.1	33.8	34.9	33.8	33.1	34.6	34.4	0.130	0.533
Shell (%)	8.80	9.00	9.05	9.18	8.90	8.96	9.10	0.608	0.156
Haugh unit	92.4	91.9	92.0	92.1	92.1	92.2	91.9	0.813	0.139
Number of eggs	23.7	24.3	25.2	24.3	23.9	24.6	23.3	0.730	0.825
Egg mass (g/bird)	10.7	11.1	11.3	10.9	10.6	11.2	10.7	0.830	0.378

[1] C=control diet; [2] W= 1% of whey powder; [3] WM= 1% whey powder + 1% dried mealworm; [4] WB= 1% whey powder + *Lactobacillus plantarum* bacteria at the rate of 4 × 10⁹ CFU/kg; [5] WP= 1% whey powder + 100g/ton Parsi Lact commercial probiotic (including *Bacillus coagulans*, *Enterococcus faecium*, *Bacillus subtilis*, *Bacillus ligiiformis*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*) in the amount of 100 grams per ton of feed; [6] MBW= 1% mealworm + *Lactobacillus plantarum* + 1% whey powder; [7] MPW= 1% mealworm + commercial probiotic + 1% whey powder; [8] Standard error of mean.

T**able 7:** The effect of experimental treatments on egg quality traits of laying quails at the average of 26 and 30 weeks of age

Parameters	Treatments								
	C[1]	W[2]	WM[3]	WB[4]	WP[5]	MBW[6]	MPW[7]	P values	SEM[8]
Egg weight (g)	12.6	12.9	12.9	12.6	12.7	12.6	12.9	0.265	0.144
Shell thickness (mm)	0.218	0.225	0.225	0.218	0.220	0.223	0.223	0.339	0.003
White height (mm)	5.05	5.06	5.05	5.04	5.05	5.06	5.03	0.965	0.03
White (%)	58.0	57.4	56.4	57.6	58.4	57.1	57.1	0.057	0.456
Yolk (%)	33.1	33.6	34.3	33.4	32.6	33.7	33.7	0.083	0.416
Shell (%)	8.93	9.45	9.32	9.46	9.08	9.20	9.17	0.103	0.143
HU	91.9	91.8	91.7	91.8	91.8	91.9	91.8	0.474	0.097
Number of eggs	47.9	50.6	50.0	49.1	48.5	52.6	48.1	0.275	1.47
Egg mass (g/bird)	10.8	11.4	11.3	10.8	10.9	11.3	11	0.668	0.307

[1] C=control diet; [2] W= 1% of whey powder; [3] WM= 1% whey powder + 1% dried mealworm; [4] WB= 1% whey powder + *Lactobacillus plantarum* bacteria at the rate of 4 × 10⁹ CFU/kg; [5] WP= 1% whey powder + 100g/ton Parsi Lact commercial probiotic (including *Bacillus coagulans*, *Enterococcus faecium*, *Bacillus subtilis*, *Bacillus ligiiformis*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*) in the amount of 100 grams per ton of feed; [6] MBW= 1% mealworm + *Lactobacillus plantarum* + 1% whey powder; [7] MPW= 1% mealworm + commercial probiotic + 1% whey powder; [8] Standard error of mean.

Intestinal morphology

The effect of the experimental treatments on the gut morphology of the laying quails is shown in Table 8. The experimental treatments had significant effects on villus length, villus width, and the villus-to-crypt ratio in the duodenum ($P< 0.05$). The shortest villi were observed in the control group, which differed significantly from all other experimental treatments except for those containing W, WM, and WP. The control group also had the shallowest crypt depth, showing a significant difference from the WM and WB treatments. The lowest villus-to-crypt ratio was recorded in the WM treatment, which differed significantly from the MBW and MPW treatments, where the highest ratios were observed. In addition, the experimental treatments significantly affected villus width in the ileum ($P< 0.05$), with the highest values observed in the WP treatment and the lowest in the WB treatment.

Blood serum metabolites

Table 9 summarizes the effects of the experimental treatments on blood serum parameters. No significant differences ($P>0.05$) were found among treatments for blood serum glucose, cholesterol, albumin, calcium, HDL-c, LDL-c, or the LDL-c: HDL-c ratio. In contrast, the treatments had significant effects on blood protein, phosphorus, and triglyceride concentrations in laying hens ($P< 0.05$). The highest protein and phosphorus concentrations were observed in the W-containing treatment, while the lowest values occurred in the MBW treatment, with a significant difference between these two groups. For blood triglycerides, the highest concentration was recorded in the treatment containing W alone, which was significantly higher than those in the control, WP, MBW, and MPW treatments.

Internal organ weights

The effects of the experimental treatments on the relative weights of internal organs are presented in Table 10. At 30 weeks of age, no significant differences ($P>0.05$) were observed in carcass yield or in the relative weights of the heart, liver, and gizzard.

Microbial culture

Table 11 presents the effects of the experimental treatments on cecal microflora. No significant differences ($P>0.05$) were observed between the experimental and control groups in the counts of *Lactobacillus* or *Escherichia coli*.

Table 8. The effect of experimental treatments on the intestinal morphology of laying quails

Treatments	Duodenum (µm)				Jejunum (µm)				Ileum (µm)			
	Villus length (V)	Crypt depth (C)	Villus width	V:C	Villus length	Crypt depth	Villus width	V:C	Villus length	Crypt depth	Villus width	V:C
C[1]	515.9 [c]	86.2 [b]	86.4	6.23 [ab]	701.9	117.8	98.2	6.02	439.6	86.03	96.82[ab]	5.18
W[2]	581.3 [abc]	115.6 [ab]	119.5	5.45 [ab]	527.7	99.9	109.7	5.44	398.9	105.6	98.0[ab]	4.13
WM[3]	557.7 [bc]	136.7 [a]	124.1	4.09 [b]	456.4	99.5	95.8	5.16	376.4	84.04	90.83[ab]	4.61
WB[4]	722.3 [a]	127.4 [a]	113.7	5.97 [ab]	849.1	102.1	112.3	9.25	428.5	89.90	86.22[b]	5.02
WP[5]	584.8 [abc]	112.3 [ab]	110.9	5.64 [ab]	678.3	115.9	102.8	6.24	469.1	107.4	124.78[a]	4.82
MBW[6]	714.9 [a]	112.6 [ab]	134.5	6.60 [a]	702.7	125.6	109.8	6.22	492.3	109.8	112.93[ab]	4.78
MPW[7]	693.1 [ab]	108.3 [ab]	198.0	6.49 [a]	720.6	109.9	101.08	6.57	480.9	112.7	99.51[ab]	4.37
P values	0.028	0.001	0.229	0.016	0.188	0.318	0.645	0.511	0.051	0.051	0.035	0.554
SEM[8]	52.9	8.95	29.48	0.510	106.4	9.27	7.61	1.43	28.94	8.13	8.58	0.400

[1] C=control diet; [2] W= 1% of whey powder; [3] WM= 1% whey powder + 1% dried mealworm; [4] WB= 1% whey powder + *Lactobacillus plantarum* bacteria at the rate of 4 × 10^9 CFU/kg; [5] WP= 1% whey powder + 100g/ton Parsi Lact commercial probiotic (including *Bacillus coagulans*, *Enterococcus faecium*, *Bacillus subtilis*, *Bacillus ligiiformis*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*) in the amount of 100 grams per ton of feed; [6] MBW= 1% mealworm + *Lactobacillus plantarum* + 1% whey powder; [7] MPW= 1% mealworm + commercial probiotic + 1% whey powder; [8] Standard error of mean.

Table 9: The effect of experimental treatments on the blood serum parameters of laying quails

Parameters (mg/dL)	Treatments								
	C[1]	W[2]	WM[3]	WB[4]	WP[5]	MBW[6]	MPW[7]	P values	SEM[6]
Glucose	278.5	251.8	247.1	259.4	253.3	242.0	272.1	0.102	9.66
Protein	4.09[ab]	4.75[a]	4.06 [ab]	4.32[ab]	4.33[ab]	3.60[b]	4.35[ab]	0.010	0.204
Albumin	1.76	1.84	1.70	1.70	1.76	1.55	1.79	0.193	0.075
Calcium	8.06	8.85	7.88	8.44	6.45	6.94	9.11	0.066	0.667
Phosphorus	7.09 [ab]	9.50[a]	8.19[ab]	6.71[ab]	6.59[ab]	5.06[b]	6.84[ab]	0.004	0.722
Triglyceride	105.0[b]	201.1[a]	139.5[ab]	130.9[ab]	97.3[b]	72.0[b]	116.0[b]	0.001	18.46
Cholesterol	156.6	190.9	168.1	166.3	159.3	141.8	161.8	0.275	13.00
LDL-c	112.9	131.9	114.1	125.1	117.3	100.9	111.2	0.568	11.15
HDL-c	22.7	18.7	29.0	25.6	23.9	26.5	27.3	0.338	0.163
LDL-c:HDL-c	5.06	7.66	4.91	5.41	5.37	4.15	4.56	0.117	0.839

[1] C=control diet; [2] W= 1% of whey powder; [3] WM= 1% whey powder + 1% dried mealworm; [4] WB= 1% whey powder + *Lactobacillus plantarum* bacteria at the rate of 4 × 10⁹ CFU/kg; [5] WP= 1% whey powder + 100g/ton Parsi Lact commercial probiotic (including *Bacillus coagulans*, *Enterococcus faecium*, *Bacillus subtilis*, *Bacillus ligiiformis*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*) in the amount of 100 grams per ton of feed; [6] MBW= 1% mealworm + *Lactobacillus plantarum* + 1% whey powder; [7] MPW= 1% mealworm + commercial probiotic + 1% whey powder; [8] Standard error of mean.

Table 10: The effect of experimental treatments on the relative organ weights of laying quails

Parameters	Treatments								
	C[1]	W[2]	WM[3]	WB[4]	WP[5]	MBW[6]	MPW[7]	P values	SEM[6]
Body weight (g)	269.1	275.8	280.6	277.4	268.4	274.1	274.2	0.423	8.46
Liver (%)	2.5	2.3	2.4	2.7	2.4	2.5	2.3	0.759	0.759
Heart (%)	0.65	0.70	0.69	0.78	0.73	0.75	0.76	0.425	0.047
Gizzard (%)	1.88	2.00	2.10	2.36	2.09	1.96	2.05	0.168	0.121

[1] C=control diet; [2] W= 1% of whey powder; [3] WM= 1% whey powder + 1% dried mealworm; [4] WB= 1% whey powder + *Lactobacillus plantarum* bacteria at the rate of 4 × 10⁹ CFU/kg; [5] WP= 1% whey powder + 100g/ton Parsi Lact commercial probiotic (including *Bacillus coagulans*, *Enterococcus faecium*, *Bacillus subtilis*, *Bacillus ligiiformis*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*) in the amount of 100 grams per ton of feed; [6] MBW= 1% mealworm + *Lactobacillus plantarum* + 1% whey powder; [7] MPW= 1% mealworm + commercial probiotic + 1% whey powder; [8] Standard error of mean.

Table 11: The effect of experimental treatments on the cecal microbial population of laying quails

Parameters	Treatments								
Log_{10} CFU/g	C[1]	W[2]	WM[3]	WB[4]	WP[5]	MBW[6]	MPW[7]	P values	SEM[8]
Lactobacillus	8.12	7.87	8.22	8.04	8.09	8.24	8.04	0.225	0.098
Escherichia oli	4.68	5.02	4.88	4.8	4.68	4.6	4.9	0.667	0.167

[1] C=control diet; [2] W= 1% of whey powder; [3] WM= 1% whey powder + 1% dried mealworm; [4] WB= 1% whey powder + *Lactobacillus plantarum* bacteria at the rate of 4 × 10⁹ CFU/kg; [5] WP= 1% whey powder + 100g/ton Parsi Lact commercial probiotic (including *Bacillus coagulans*, *Enterococcus faecium*, *Bacillus subtilis*, *Bacillus ligiiformis*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*) in the amount of 100 grams per ton of feed; [6] MBW= 1% mealworm + *Lactobacillus plantarum* + 1% whey powder; [7] MPW= 1% mealworm + commercial probiotic + 1% whey powder; [8] Standard error of mean.

Discussion

The proximate composition of mealworm revealed a predominance of crude protein (52.61%), followed by ether extract (28.3%), crude fiber (7.43%), crude ash (6.78%), and phosphorus (6.60%). These values align with previously reported ranges for crude protein (47–60.2%), ether extract (19.1–36.7%), and crude ash (2.65–6.99%) (Hong *et al.*, 2020). However, the crude fiber content observed in this study (7.43%) was lower than that reported by Kröncke and Benning (2023), who used specific agricultural by-products as feed. This variation underscores the influence of feed composition and rearing conditions on the nutritional profile of mealworms.

A consistent and significant reduction in FI was observed in several whey-containing treatments (W, WB, MBW, MPW), particularly during the later stages of the trial (weeks 26–30) and across the overall experimental period (weeks 22–30). Importantly, this decrease did not compromise productive performance, indicating improved feed utilization efficiency in these groups. This aligns with

previous findings that probiotics and prebiotics can enhance nutrient digestibility and absorption, allowing birds to maintain production on reduced FI (Naeem and Bourassa, 2025). For example, Hilmi *et al.* (2020) reported that fermented whey supplementation lowered FI while improving FCR in laying hens, and Ashour *et al.* (2019) observed similar outcomes in broilers fed whey protein concentrate. These effects may partly arise from the satiety-inducing properties of whey proteins, which stimulate the secretion of gut hormones such as cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and peptide YY (PYY) (Hall *et al.*, 2003). Hall *et al.* (2003) further demonstrated that whey ingestion suppressed appetite more effectively than casein, with GLP-1 levels showing a strong inverse correlation with desire to eat, providing a plausible physiological mechanism for the present findings.

Feed palatability may also contribute to reduced FI. Poultry are sensitive to the organoleptic qualities of feed, and whey—particularly acid whey with high glucose–lactose content and a sour flavor—can be less palatable (Neves *et al.*, 2014; Das *et al.*, 2025), potentially decreasing voluntary intake in some treatments.

In mealworm-containing diets, reduced FI may be linked to the chitin content of insect meals, which can lower crude protein and organic matter digestibility due to the limited chitinase activity in poultry (Belhadj Slimen *et al.*, 2023). Shariat Zadeh *et al.* (2020) similarly reported decreased FI when fish meal was fully replaced with mealworm powder in laying quails. However, results across studies are inconsistent. For example, Dalle Zotte *et al.* (2024) found that a 10% inclusion of live mealworm increased FI in laying quails but did not significantly improve production performance, while Sedgh-Gooya *et al.* (2021) observed that a 2.5% inclusion of mealworm meal improved EP and FCR without affecting FI in laying hens.

The effects of probiotics and synbiotics on FI in quails are likewise variable. Abou-Kassem *et al.* (2021) reported no changes in FI or FCR with *Bacillus toyonensis* and *Bifidobacterium bifidum* supplementation, and Jazi *et al.* (2020) found that whey, *Bacillus subtilis*, or their combination did not alter FI despite improving FCR. Similarly, Hamzehee *et al.* (2025) reported no significant differences in EP or FCR in laying hens when *B. coagulans* was combined with whey powder. In contrast, Siadati *et al.* (2018) observed that local *Lactobacillu*s strains improved FCR without affecting FI, and Tufan and Bolacali (2017) found that synbiotic supplementation enhanced performance in Japanese quail. These discrepancies likely reflect differences in probiotic strains, dosages, insect meal inclusion levels, dietary composition, and bird genotype, as well as potential synergistic or antagonistic interactions between feed additives.

The present study found no significant differences in egg quality traits between the control and experimental groups. This agrees with previous research indicating that dietary inclusion of insect-based meals or probiotics often has little effect on parameters such as egg weight, shell thickness, or HU. For example, Sedgh-Gooya *et al.* (2021) reported that replacing dietary protein with varying levels of mealworm did not affect shell thickness, shell percentage, or HU in laying hens. Similarly, Manafi *et al.* (2016) observed no changes in shell thickness or HU in laying quails supplemented with *Bacillus subtilis*, and Saksrithai *et al.* (2019) found no significant effects of *Lactobacillus* supplementation on egg quality in White Leghorn hens. Hamzehee *et al.* (2025) also reported no significant interaction between whey powder and *Bacillus coagulans* for these traits. Although some studies have noted numerical improvements in egg weight, shell thickness, and HU with whey powder or synbiotic supplementation (Aghaei *et al.*, 2010; Tufan and Bolacali, 2017), such effects are often not statistically significant. Differences in findings among studies may reflect variation in bird age, species or strain, environmental and management conditions, dietary nutrient composition, additive inclusion levels, and feeding duration.

The present findings align with previous research showing that probiotics and prebiotics can enhance intestinal architecture in poultry by increasing villus height and, in some cases, modifying crypt depth, thereby improving nutrient absorption efficiency (Biswas *et al.*, 2018; Aruwa *et al.*, 2021). In our study, the increase in duodenal villus height in whey-containing treatments may be attributed to the high concentrations of branched-chain amino acids and bioactive peptides in whey proteins, which are known to support epithelial integrity and gut barrier function (Idowu *et al.*, 2025). Probiotics have also been shown to stimulate crypt cell proliferation (He *et al.*, 2019), an essential process for epithelial renewal and nutrient assimilation. Mechanistically, these morphological enhancements may result from the synergistic effects of whey and probiotics: whey lactose acts as a fermentable substrate for beneficial bacteria, leading to SCFA production, which in turn promotes enterocyte proliferation and villus elongation (Smith *et al.*, 2020). A higher villus height-to-crypt depth ratio is widely recognized as an indicator of improved intestinal health, as shallower crypts reflect reduced epithelial turnover and a greater allocation of nutrients toward production (Tsiouris *et al.*, 2020).

Comparable results have been reported in Japanese quail, where native *Lactobacillus* strains increased villus height and the villus height-to-crypt

depth ratio (Siadati *et al.,* 2018). Similarly, *Bacillus coagulans* supplementation alone has been associated with increased ileal villus width (Hamzehee *et al.,* 2025). However, findings across studies are not always consistent. For example, *Bacillus subtilis* supplementation in quail has been linked to reductions in both villus height and crypt depth (Manafi *et al.,* 2016), and the combination of whey powder with *Bacillus subtilis* in broiler chickens produced no significant morphological changes in the duodenum, jejunum, or ileum except for an improved ileal villus height-to-crypt depth ratio (Jazi *et al.,* 2020). The inclusion of mealworm meal has also produced variable outcomes. Some studies have reported increases in villus height and crypt depth (Zadeh *et al.,* 2019), while others found no morphological changes in broilers (Biasato *et al.,* 2018) or laying hens (Sedgh-Gooya *et al.,* 2022). Such discrepancies may be explained by differences in probiotic strain and viability, nutrient composition and chitin content of the insect meal, additive inclusion levels, bird species and age, or the duration of feeding trials.

An unexpected outcome in our study was the deeper crypt depth observed in WM and WB treatments, whereas the control group exhibited the shallowest crypts. As probiotics are often associated with reduced crypt depth, this finding is counterintuitive (Idowu *et al.,* 2025). One possible explanation is that the high-protein substrates provided by whey and insect meal increased enterocyte turnover, necessitating deeper crypts to sustain elevated absorptive demand (Rubin and Levin, 2016).

Most serum metabolites measured (glucose, cholesterol, HDL-c: LDL-c, calcium, albumin) were unaffected by supplementation, indicating that the tested feed additives did not disrupt general metabolic homeostasis. This finding aligns with earlier reports of limited effects of probiotics, prebiotics, or insect meals on most blood parameters in laying quails (Shariat Zadeh *et al.,* 2020; Sedgh-Gooya *et al.,* 2021; Hamzehee *et al.,* 2025).

In contrast, whey powder markedly increased total protein and phosphorus concentrations, reflecting its high content of bioavailable proteins and minerals (Madureira *et al.,* 2007). Whey is rich in branched-chain amino acids, particularly Lucien, which activates the mTOR pathway and stimulates hepatic protein synthesis (Ashour *et al.,* 2019). These effects may have been reinforced by probiotic-associated improvements in gastrointestinal secretory activity, enhancing digestion and nutrient absorption (Derakhshan *et al.,* 2023). Previous studies using lower whey inclusion rates (0.1%) in laying hens found no effect on plasma protein (Hamzehee *et al.,* 2025), suggesting that the higher level applied here facilitated greater amino acid utilization and digestibility. Comparable results have been observed in broilers, where whey protein concentrate supplementation increased serum total protein compared with controls (Szczurek *et al.,* 2013).

Phosphorus levels followed a similar trend, with whey diets producing the highest values. This likely reflects whey's phosphoprotein and phosphopeptide content and the close metabolic coupling of protein and phosphorus utilization (Madureira *et al.,* 2007; Tsiouris *et al.,* 2020). Enhanced mineral bioavailability via probiotic activity may have contributed to this effect (Szczurek *et al.,* 2013). These findings contrast with studies reporting no significant influence of probiotics or mealworm meal on serum phosphorus (Tang *et al.,* 2017; Shariat Zadeh *et al.,* 2020).

Conversely, the MBW treatment resulted in the lowest protein and phosphorus concentrations, suggesting antagonistic interactions between components. Chitin in mealworm meal can reduce protein digestibility, while probiotic-induced changes in gut pH and enzyme activity may further limit nitrogen absorption (Stastnik *et al.,* 2021). Additionally, organic acids from *Lactobacillus plantarum* fermentation can chelate phosphorus, reducing its bioavailability despite potential gut health benefits.

Whey powder alone produced the highest plasma triglyceride concentrations, in contrast to the lipid-lowering effects typically associated with probiotics and prebiotics (Sharifi *et al.,* 2011; Jazi *et al.,* 2020). This increase may arise from whey's lactose and fat content stimulating hepatic lipogenesis and very low-density lipoprotein (VLDL) secretion, or from elevated yolk lipid synthesis driven by improved protein availability (Ito *et al.,* 2003). Adequate protein supports vitellogenin production—a triglyceride-rich yolk precursor—thereby increasing plasma triglyceride in parallel with reproductive activity (Ito *et al.,* 2003). Furthermore, whey's rapid digestibility and high branched-chain amino acid content can stimulate insulin secretion, which under stable carbohydrate intake, promotes lipogenesis (Madureira *et al*., 2007).

By contrast, diets combining whey with probiotics markedly reduced triglyceride concentrations. This is consistent with evidence that *Lactobacillus plantarum* produces SCFA capable of activating AMP-activated protein kinase, promoting fatty acid oxidation and suppressing lipogenesis (Cao *et al.,* 2023). Similar reductions in triglycerides have been reported in laying hens with *Lactobacillus* supplementation (Alaqil *et al.,* 2020), although other studies found no changes following probiotic or synbiotic use (Mohammadian *et al.,* 2013; Mohebbifar *et al.,* 2013). Such discrepancies may reflect differences in probiotic strain, mealworm

composition, dosage, bird species, or physiological stage.

In the present study, no significant differences were detected in carcass yield or the relative weights of the heart, liver, and gizzard at 30 weeks of age. These findings are consistent with a previous report indicating that mealworm larvae inclusion does not alter carcass characteristics in poultry (Zadeh *et al.,* 2019). Prebiotic supplementation, alone or in combination with probiotics, has also been shown to have minimal impact on organ weights. Samli *et al.* (2007) and Ashayerizadeh *et al.* (2009) both reported no significant differences in the weights of the liver, heart, or proventriculus when birds were fed diets containing prebiotics or synbiotics, aligning with the present results. These consistent findings across multiple studies suggest that the tested dietary interventions, including insect-based meals, prebiotics, and synbiotics, do not adversely affect carcass yield or the relative development of key internal organs in poultry (Islam and Yang, 2017; Tufan and Bolacali, 2017).

In the present study, cecal *Lactobacillus* and *Escherichia coli* populations did not differ significantly between control and treated groups. This contrasts with numerous reports in quails where probiotic supplementation increased beneficial *Lactobacillus* counts while reducing pathogenic bacteria such as *Escherichia coli* and *Salmonella* (Siadati *et al.,* 2018; Sultan *et al.,* 2024). The absence of such effects in the present trial may be attributed to strain-specific efficacy, insufficient colonization time, suboptimal dosage, or possible antagonistic interactions among the multiple additives administered in combination. Supporting this possibility, Jazi *et al.* (2020) reported increased lactic acid bacteria counts in quails fed whey, *Bacillus subtilis*, or their combination, suggesting that additive-specific synergies can occur under certain dietary contexts.

Edible insects such as mealworms and black soldier fly have been reported to modulate cecal microbiota modestly, an effect often linked to the antimicrobial and immunostimulatory properties of chitin (Islam and Yang, 2017; Colombino et al., 2021). However, the mealworm powder used in the present study contained only 5.6% chitin, which may have provided insufficient substrate to induce measurable microbial shifts. The lack of a significant reduction in *Escherichia coli* counts is consistent with earlier studies showing that insect-based diets, including up to 5% mealworm meal, did not markedly affect *Escherichia coli* levels in laying birds (Hajati and Negarandeh, 2021; Stastnik *et al.,* 2021).

Conclusion

The present study demonstrated that dietary inclusion of prebiotic and probiotic sources, either individually or in combination, did not significantly affect EP, FCR, egg quality traits, carcass yield, or the relative weights of internal organs in laying quails. However, several treatments, particularly those containing whey powder, resulted in reduced FI compared with the control. At the metabolic level, whey supplementation was associated with elevated serum protein, phosphorus, and triglyceride concentrations, whereas combinations with probiotics and prebiotics appeared to mitigate these lipid increases. Improvements in intestinal morphology were also observed, as experimental diets enhanced duodenal villus length, villus width, and the villus-to-crypt ratio, suggesting potential benefits for nutrient absorption and gut health. In contrast, cecal microbial populations were not significantly altered. Overall, these findings indicate that functional feed additives such as whey, insect meals, and probiotics may contribute to intestinal morphology and metabolic regulation in laying quails, although their impact on production performance appears limited. Future research is needed to establish optimal inclusion levels, effective additive combinations, and appropriate feeding durations, while also assessing the economic feasibility of these strategies under commercial production conditions to determine their practical value.

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